Amendments to the Specification

Please amend the specification as follows:

On page 1, line 1, please substitute the following title:

VACCINEOUTER MEMBRANE VESICLES AND USES THEREOF

On page 7, beginning on line 20, please substitute the following paragraph:

The kanamycin-resistance cassette (KAN) replaces *msbA* in the mutant, leaving only 131 bp at the 3' end (M). Primers used for the disruption procedure and cloning of *msbA* are indicated with arrows. Primer sequences are (A) CCCAAAGCGAAGTGGTCGAA (SEQ ID NO: 6); (B) GTCGACTATCGGTAGGGCGGGAACTG (AccI restriction site is underlined) (SEQ ID NO: 7); (C) GTCGACGACCGCATCATCGTGATGGA (AccI restriction site is underlined) (SEQ ID NO: 8); (D) TTCGTCGCTGCCGACCTGTT (SEQ ID NO: 9); (E) TTCATATGATAGAAAAACTGACTTTCGG (NdeI restriction site is underlined) (SEQ ID NO: 10); (F) GACGTCCCATTTCGGACGGCATTTTGT (ActII restriction site is underlined) (SEQ ID NO: 11). Predicted promoter (P) and terminator (T) sequences are indicated. ORFs indicated with NMB1918 and NMB1920 putatively code for a malonyl CoA-acyl carrier protein transacylase and GMP synthase, respectively.

On page 14, beginning on line 4, please substitute the following paragraph:

Further Neisserial OMP loops that may be substituted for Imp loops (particularly loops 3 and/or 8) are PorA loop 4 [or variable region 2]-(see http://neisseria.org/nm/typing/porA/); PorA loop 5 (described in "Topology of outer membrane porins in pathogenic Neisseria spp", van der Ley, Poolman, etc.., Infect Immun 1991, 59, 2963-71; [[]]its sequence in PorA P1.7,16 (H44/76) loop 5 being: RHANVGRNAFELFLIGSGSDQAKGTDPLKNH, SEQ ID NO: 12); LbpA surface exposed loops 4, 5, 7, 10 and 12, corresponding to amino acids 210-342, 366-441, 542-600, 726-766 and 844-871, respectively, with 12 being preferred (sequence KGKNPDELAYLAGDQKRYSTKRASSSWST), SEQ ID NO: 13) [see Prinz et al.

1999 J Bacter. 181:4417 for further details on LbpA surface loops incorporated by reference herein]; NspA surface exposed loops 1, 2, 3 or 4, corresponding to amino acid sequence 25-54, 61-87, 103-129 and 149-164, respectively, preferably where loop 2 (e.g. FAVDYTRYKNYKAPSTDFKLYSIGASA, SEQ ID NO: 14) and/or 3 (e.g. ARLSLNRASVDLGGSDSFSQTSIGLGVL, SEQ ID NO: 15) is inserted (as these loops are quite small not all the Imp loop 2 and/or 8 would be ideally removed to introduce these loops, and if both are to be introduced, it is preferred that they are introduced on loop 2 or 8 (or vice versa) in order to try to preserve the conformational epitope that exists between loops 2 and 3 of NspA) [see Vandeputte-Rutten et al 2003 JBC 278:24825 for more details on NspA loops, incorporated by reference herein]; any of the surface exposed loops of Omp85 (see Science 2003 299:262-5, and supporting online material Fig S4, incorporated by reference herein).

On page 30, beginning on line 16, please substitute the following paragraphs:

A preferred oil-in-water emulsion comprises a metabolisible oil, such as squalene, alpha tocopherol and Tween 80 TWEEN® 80 (polysorbate 80). In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of $1\mu g$ - $200\mu g$, such as $10\text{-}100\mu g$, preferably $10\mu g$ - $50\mu g$ per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3%-tween 80 TWEEN® 80 (polysorbate 80). Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80 TWEEN® 80 (polysorbate 80), in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

On page 46, beginning on line 30, please substitute the following paragraph:

Neisseria meningitidis encodes two RTX proteins, referred to as FrpA & FrpC secreted upon iron limitation (Thompson *et al.*, (1993) J. Bacteriol. 175:811-818; Thompson *et al.*, (1993) Infect. Immun. 61:2906-2911). The RTX (Repeat ToXin) protein family [[]]have in common a series of 9 amino acid repeat near their C-termini with the consensus: Leu Xaa Gly Gly Xaa Gly (Asn/Asp) Asp Xaa, SEQ ID NO: 16 / SEQ ID NO: 30. (LXGGXGN/DDX). The repeats in *E. coli* HlyA are thought to be the site of Ca2+ binding. As represented in Figure 4, meningococcal FrpA and FrpC proteins, as characterized in strain FAM20, share extensive amino-acid similarity in their central and C-terminal regions but very limited similarity (if any) at the N-terminus. Moreover, the region conserved between FrpA and FrpC exhibit some polymorphism due to repetition (13 times in FrpA and 43 times in FrpC) of a 9 amino acid motif.

On page 48, beginning on line 21, please substitute the following paragraph:

This process can advantageously enhance the stability and/or immunogenicity (providing T-cell help) and/or antigenicity of the LOS antigen within the bleb formulation — thus giving T-cell help for the T-independent oligosaccharide immunogen in its most protective conformation — as LOS in its natural environment on the surface of meningococcal outer membrane. In addition, conjugation of the LOS within the bleb can result in a detoxification of the LOS (the Lipid A portion being stably buried in the outer membrane thus being less available to cause toxicity). Thus the detoxification methods mentioned herein of isolating blebs from htrB or msbB mutants, or by adding non toxic peptide functional equivalent of polymyxin B [a molecule with high affinity to Lipid A] to the composition (see WO 93/14115, WO 95/03327, Velucchi et al (1997) J Endotoxin Res 4: 1-12, and EP 976402 for further details of non-toxic peptide functional equivalents of polymyxin B — particularly the

use of the peptide SAEP 2 (of sequence KTKCKFLKKC, SEQ ID NO: 17, where the 2 cysteines form a disulphide bridge)) may not be required (but which may be added in combination for additional security). Thus the inventors have found that a composition comprising blebs wherein LOS present in the blebs has been conjugated in an intra-bleb fashion to outer membrane proteins also present in the bleb can form the basis of a vaccine for the treatment or prevention of diseases caused by the organism from which the blebs have been derived, wherein such vaccine is substantially non-toxic and is capable of inducing a T-dependent bactericidal response against LOS in its native environment.

On page 61, beginning on line 29, please substitute the following Table 1:

Table 1. Oligonucleotides (primers) used in this study. Underlined sequences indicate restriction sites: *AccI* in primers B, C, E and F; *N*deI in primers G and H, *AatII* in primer D and *BamHI* in primer I. Dashed line in primer F indicates the Neisserial DNA uptake sequence.

	Sequence (5'-3')	Purpose
A	ATGCCTGCAACCTTCAAGTG, SEQ ID	5' primer for cloning of NMB0279
	NO: 18	
В	ATGTCGACAATCGCCCCTCAAGTCGGTT	3' primer for cloning of_NMB0279
	TG, SEQ ID NO: 19	
C	ATGTCGACTACCTGCGGCCGGATTATGC	5' primer for cloning of 3'
	, SEQ ID NO: 20	end of imp
D	ATGACGTCTCAGGGTCGTTTGTTGCGTC	3' primer for cloning of 3'
	CGGC <u>, SEQ ID NO: 21</u>	end of imp
Е	AGCGTCGACTTCAGACGGCCACGTTGTG	5' primer for cloning of
	TC, SEQ ID NO: 22	Kan-cassette
F	AGCGTCGACGCTGAGGTCTGCCTCGTG,	3' primer for cloning of
	SEQ ID NO: 23	Kan-cassette
G	ATCATATGGCTCGTTTATTTTCACTCAA	5' primer for cloning of

Application No. 10/584,362 Attorney Docket No. VB60639

	ACC, SEQ ID NO: 24	complete imp gene into pEN11
H	TGCATATGGATGCCGTTGCGGCGGAG,	5' primer for cloning of
	SEQ ID NO: 25	imp into pET11a
I	TGGGATCCTCAGGGTCGTTTGTTGCGTC	3' primer for cloning of
	C, SEQ ID NO: 26	imp into pET11a